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Vaccine characterization of Patients with Metastatic Breast Cancer
Department of Defense Grant # DAMD17-03-1-0487
Annual Progress Report, July 2012
[For the period of 07/01/2011 - 06/30/12]

David Avigan, MD
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Introduction

The main objective of the study is to determine the safety, immunologic response, and clinical effect of vaccination with dendritic cell (DC)/breast cancer fusions administered in conjunction with IL-12 to patients with metastatic breast cancer. DC/breast carcinoma fusion cells present a broad array of tumor-associated antigens in the context of DC-mediated costimulation. In clinical studies, vaccination with fusion cells was well tolerated, induced immunologic responses in a majority of patients, and resulted in disease regression in subset. We postulated that administration of the vaccine in conjunction with IL-12 would further enhance vaccine response by promoting T cell activation. In this study, we are conducting a phase I/II clinical trial in which patients with metastatic breast cancer undergo vaccination with DC/tumor fusions administered in conjunction with rhIL-12. An initial cohort of patients was treated with the DC/tumor fusions alone. In the subsequent cohort, fusion cells are being administered with rhIL-12 given subcutaneously at the time of vaccine administration and on days 3 and 5. Measures of tumor specific cellular and humoral immunity are being obtained at serial time points following vaccination. Time to disease progression and RECIST measurable disease response will be followed as a secondary endpoint.

Clinical Trial

After completion of a review by the DOD, IRB and the FDA, patients were enrolled in the activated protocol for the generation of DC/tumor fusion vaccines. As reported in the Quarterly Reports of July 2011 through March 2012, seven patients have been enrolled thus far. We reported in detail the characterization of the tumor cells, the generated dendritic cells and the DC/tumor fusions pertaining to Patients BV01, BV03, BV04, BV05 and BV06. Patient BV07 has had her tumor collected and is being characterized at the time of writing this report. Of the seven participants enrolled (as of July 2012), three came off study prior to receiving the investigational vaccine: two had progressive disease, and one elected to pursue standard of care therapy only. Three participants, BV01, BV04 and BV06, completed vaccinations. Two patients demonstrated disease progression during the follow up period.

Since the previous updates, patient BV07 was enrolled in the study after meeting all eligibility criteria. Participant BV07 had her tumor collection on July 2nd, 2012. Characterization of the collected tumor cells is listed in Table 1. Preparation of the tumor-DC fusion vaccine is currently in progress.

Summary of patients processed for the study for the period of 07/01/2011 - 06/30/12

Patients recruited for the study for the above period are summarized below. Table 1 lists the properties of tumor cells isolated from the patients (from pleural effusion and tissue biopsy)

before and after cryopreservation. Table 2 lists the characterization of dendritic cells generated from leukapheresis collection.

Isolation of tumor cells: Table 1 below summarizes the properties of tumor cells isolated from the patients before and after cryopreservation.

	Tumor properties at times of collection		**Properties of cryopreserved tumor cells after thawing**	
Patient #	Total yield of tumor cells	Viability at time of isolation	Total yield	Viability
BV01	$93x10^6$	99%	$85x10^6$	88%
BV03	$10x10^6$	84%	ND	ND
BV04	$1220x10^6$	99%	$336x10^6$	92%
BV05	$58x10^6$	95%	$42.6x10^6$	88%
BV06	$84x10^6$	86%	$21x10^6$	76%
BV07	$93x10^6$	84%	ND	ND

Table 1. *Tumor cells were isolated and excess RBCs lysed in the presence of ammonium chloride. Tumor cells were cultured in RPMI 1640 containing gentamycin, human insulin and 10% autologous plasma at 37°C. An aliquot of cells was subjected to immunohistochemical staining. Before fusion, an aliquot of tumor cells was harvested to generate tumor lysate for* in vitro *testing. The tumor cell yields and viability are presented at the time of harvesting cells and following cryopreservation.*

Generation of dendritic cells:

All patients listed below, underwent a single leukapheresis collection. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque and cultured in RPMI 1640 with 1% autologous plasma for 1–2 h to select the adherent cell population. Nonadherent cells were removed, and the adherent population of cells was cultured in complete RPMI 1640 containing 1% autologous sera. The yields and viability of the cells following leukapheresis and the generation of dendritic cells (and phenotypic surface markers) are listed in Table 2.

Patient #	Cell yield from leukapheresis collection	Percentage recovery of cells after Ficoll	Number of DCs generated from adherent cells	Viability of DCs following 7-10 day culture	DC positive markers
BV01	$1x10^9$	57%	$274x10^9$	92%	HLA-DR, CD11c, CD86
BV04	$5.32x10^9$	52%	$85x10^6$	87%	HLA-DR, CD11c, CD86
BV05	$4.17x10^9$	54%	$56x10^6$	90%	HLA-DR, CD11c, CD86
BV06	$7.73x10^9$	48%	$68x10^6$	89%	HLA-DR, CD11c, CD86

Table 2. *Patients underwent a single leukapheresis collection. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque density centrifugation and cultured in RPMI 1640 with 1% autologous plasma for 1–2 h. Nonadherent cells were removed, and the adherent population was cultured in RPMI 1640 containing gentamicin, 1% autologous sera, 500 units/ml recombinant human interleukin IL-4 and 1000 units/ml GM-CSF for 7–10 days. An aliquot of cells from each preparation was removed for immunocytochemical and functional analysis. Cell yields from the leukapheresis collection and after Ficoll centrifugation, and the number of dendritic cells generated from adherent cells are presented. Surface phenotypic markers of DCs generated are also depicted.*

Tumor cells and DCs were combined at a ratio of 1:7 and washed in serum-free medium. After low-speed centrifugation, the cell pellets were resuspended in 500 µl of 50% polyethylene glycol in Dulbecco's PBS without Ca^{2+} and Mg^{2+}, washed free of polyethylene glycol, and cultured in RPMI 1640 with 10% autologous plasma and 500 IU/ml GM-CSF.
DC, tumor cells and fusion cells were stained with primary murine monoclonal antibodies against MUC1, Cytokeratin CAM, HLA-DR, CD54, or CD86 and an isotype-matched negative control for 60 min. The cells were incubated with a biotinylated $F(ab')_2$ fragment of horse antimouse IgG for 45 min and incubated for 30 min with avidin biotin complex reagent solutions followed by 3 amino-9-ethyl carbazole solution. Detection of MUC1, CAM, or cytokeratin with the avidin-biotin complex reagents was followed by staining for other markers with the avidin-biotin complex-alkaline phosphatase kit. For patient BV04, tumor cells were shown to strongly express cytokeratin, CAM and MUC1 and did not express CD86. Tumor cells from patient BV05 strongly expressed DR and CT. For patient BV06, analysis of the pleural effusion tumor cells showed >20% of cells strongly expressing cytokeratin and MUC1 (grade 4+), whereas tumor cells from tissue biopsy showed >75% of the cells intensely positive for cytokeratin and MUC1. DCs generated from all study patients strongly expressed CD86, HLA-DR, and CD11C and did not express cytokeratin, CAM or MUC1 (Table 2). Fusions generated for patients for the period of 07/01/2011 - 06/30/12 are summarized in Table 3. The fusion viability, together with fusion efficiency and the total number of doses prepared are listed in Table 3. Doses are based on the number of viable fusion cells (as assessed by trypan blue exclusion) that coexpress DC- and tumor-associated markers (see below). An adequate yield of fusion cells and the absence of contamination as assessed by assays for sterility, endotoxin, and *Mycoplasma* were completed prior to vaccination.

Patient #	Fusion double stain markers	Fusion viability	Fusion efficiency	Total Fusion Dose prepared	Number of Doses prepared
BV01	CD86/CT	82%	40%	$5x10^6$	3
BV04	CD86/CT	88%	25%	$5x10^6$	3
BV05	CD86/CT	81%	16%	$2x10^6$	3
BV06	CD86/CT	89%	28%	$3.5x10^6$	3

Table 3. Characterization of DC/tumor fusion cells*. DC/tumor fusion cells were prepared with polyethylene glycol as described above. An aliquot of the fusion cells were prepared for viability assessment and immunohistochemistry. Trypan blue analysis was used to determine the viability of fusion cells. Fusion efficiency was quantified by dual staining for the CD86/CT (cytokeratin) marker.*

Immune monitoring:

For patient BV01, vaccination resulted in the expansion of breast cancer reactive T cells as manifested by the percent of CD4 and CD8+ T cells expressing IFNγ following ex vivo exposure to autologous tumor lysate. Furthermore, weak responses to PHA and Tetanus Toxoid were observed post vaccination; whereas, no significant change was observed in the levels of regulatory T cells or those that expressed FOXP3 pre- and post-vaccination. Immunologic monitoring of the other treated patients is ongoing.

Conclusion:
The first cohort in which patients undergo vaccination with DC/tumor fusions alone has been completed. Enrollment into the second cohort in which patients receive the vaccine in conjunction with IL-12 has been initiated. Enrollment into the Phase I portion of the study will be completed in the next year.